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### Abstract

Despite their common occurrence in Guadeloupe, little is known about levels and effects of pollutants in free-ranging green (*Chelonia mydas*) and hawksbill (*Eretmochelys imbricata*) turtles. The aims of this study were (1) to evaluate levels of persistent organic pollutants and trace elements in Guadeloupian marine turtles, (2) to assess the risk for turtle embryo facing chemical exposure. Eggs and dermis were collected from 11 green and 4 hawksbill turtles and analysed for inorganic and organic pollutants. Chemical risks were evaluated for turtle embryos through a screening risk assessment (SRA).  $\Sigma\Sigma$ PCBs and chlordecone were the main contaminant groups in green and hawksbill turtles. Contaminant levels were lower in the tissues of the Guadeloupean turtles compared to other geographic locations. p,pp,p'-DDE, selenium, mercury and cadmium could affect the marine turtle embryos. This study is the first to provide levels of pollutants in marine turtles from Guadeloupe.

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# Pollutant exposure in green and hawksbill marine turtles from the Caribbean region

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## HIGHLIGHTS

- This is the first report of pollutant levels in marine turtles from Guadeloupe.
- Several pollutants were analysed in eggs and dermis in green and hawksbill turtles.
- $\Sigma$ PCBs and chlordecone were the main contaminant groups in both sea turtle species.
- Pollutant levels were lower in Guadeloupean turtles compared to other areas.
- Screening Risk Assessment suggest risk for turtle embryos.

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## ABSTRACT

Despite their common occurrence in Guadeloupe, little is known about levels and effects of pollutants in free-ranging green (*Chelonia mydas*) and hawksbill (*Eretmochelys imbricata*) turtles. The aims of this study were (1) to evaluate levels of persistent organic pollutants and trace elements in Guadeloupean marine turtles, (2) to assess the risk for turtle embryo facing chemical exposure. Eggs and dermis were collected from 11 green and 4 hawksbill turtles and analysed for inorganic and organic pollutants. Chemical risks were evaluated for turtle embryos through a screening risk assessment (SRA).  $\Sigma$ PCBs and chlordecone were the main contaminant groups in green and hawksbill turtles. Contaminant levels were lower in the tissues of the Guadeloupean turtles compared to other geographic locations. *p*, *p'*-DDE, selenium, mercury and cadmium could affect the marine turtle embryos. This study is the first to provide levels of pollutants in marine turtles from Guadeloupe.

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## 1. Introduction

The seven species of marine turtles have been listed on the IUCN Red List of threatened species (IUCN, 2014). Conservation programmes have generally focused on direct threats (e.g. fishery by-catch, poaching of eggs and harvesting of nesting females) while risks associated with environmental pollutants have received little attention.

Green (*Chelonia mydas*) and hawksbill (*Eretmochelys imbricata*) marine turtles are the most frequently encountered turtle species

in Guadeloupe (French West Indies FWI, Fig. 1) where individuals of multiple life stages have been observed in both feeding and nesting grounds (Meylan, 1983). Although their legal protection in Guadeloupe since 1991 has allowed for the recovery of some populations, the size of turtle populations in French Indies remains far below their historical levels (Chevalier et al., 2011; Troëng and Rankin, 2005).

Besides being known for hosting nesting marine turtles, the Guadeloupean archipelago has been infamous for the inappropriate use of organohalogen compounds (OHCs) including dichlorodiphenyltrichloroethane (DDT), hexachlorocyclohexane mixtures (HCHs) and, especially, chlordecone. Chlordecone was first produced as Kepone<sup>TM</sup> in the US from 1958 to 1976

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and then as Curlone™ in Brazil from 1981 to 1993 (Information, 2009). While the use of chlordecone has been banned since 1993, the compound is still being detected in the local environment (i.e. soils, rivers, spring water; drinking water and foodstuffs), posing a significant risk for wildlife and for Guadeloupean inhabitants (Cabidoche et al., 2009; Coat et al., 2011; Multigner et al., 2010). Threat of pollutant exposure, including chlordecone, towards Guadeloupean marine turtle populations has not yet been assessed.

Early stages of life in marine turtles are highly vulnerable: chemical exposure is believed to be an additional stress to juveniles already facing a high mortality rate linked to low hatching success, high predation rates and occasional poaching (Hamlin and Guillette, 2011; Seminoff and Shanker, 2008). Previous studies have suggested the use of a screening risk assessment (SRA) approach for evaluating the embryotoxicity of contaminants in marine turtles (Lam et al., 2006; Van de Merwe et al., 2009). By comparing the measured egg concentration (MEC) of a given compound to reference values, these studies have indicated that selenium and lead may affect the growth and survival of marine turtle embryos (Lam et al., 2006; Van de Merwe et al., 2009).

The present study intended to provide the first baseline concentrations of organohalogen compounds (OHCs) (chlordecone, polychlorobiphenyls PCBs, hexachlorocyclohexane isomers HCHs, dichlorodiphenyltrichloroethane and metabolites DDTs, polybrominated diphenyl ethers PBDEs) and trace elements (mercury T-Hg, lead Pb, cadmium Cd, selenium Se, copper Cu, iron Fe, zinc Zn) in green and hawksbill marine turtles nesting in Guadeloupe Island. First baseline levels were also provided for OHCs in hawksbill turtle eggs and for chlordecone in marine turtle tissues (i.e. eggs and dermis). The risk from this pollutant exposure for embryo survival was further evaluated through SRA.

## 2. Materials and methods

### 2.1. Sample collection

The study was conducted in Guadeloupe (FWI, East Wider Caribbean Region) from August to September 2008 in collaboration with Kap'Natirel Organization. The beaches of Marie-Galante and the Petite-Terre Nature Reserve (Fig. 1) were patrolled at night and surveyed for nesting females of green (*Chelonia mydas*) and hawksbill (*Eretmochelys imbricata*) marine turtles. The curved carapace length (CCL) was recorded using a flexible tape measure (Bolten, 1999). Three eggs and one dermis sample were collected from each encountered green ( $n = 11$ ) and hawksbill ( $n = 4$ ) marine turtle. Eggs were collected at the time of oviposition, rinsed with deionized water and wrapped into plastic bags. After the application of local anaesthetic spray (xylocaine 10%, spray), dermis samples were collected from the females' right shoulder with a biopsy punch (5 mm in diameter, Kai Europe GmbH, Germany) and the area was then disinfected with alcohol. Dermis samples were placed in Eppendorf tubes. All samples were kept in a portable cooler at the time of the beach patrol and then in a freezer ( $-20\text{ }^{\circ}\text{C}$ ) until analysis.

### 2.2. Ethics statement

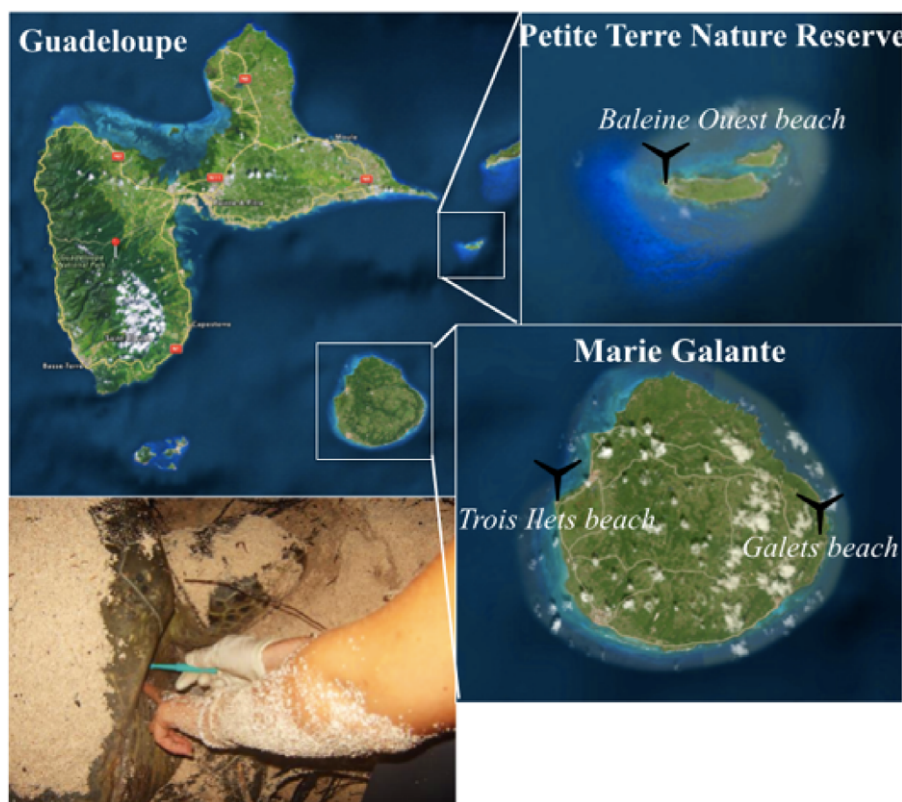
Turtle sampling and transport were authorized by the French (i.e. Direction Régionale de l'Environnement Guadeloupe, DIREN, CERFA: 11631\*01) and Belgian authorities (i.e. SPF Santé publique, Sécurité Chaîne alimentaire et Environnement, Organe de Gestion C.I.T.E.S., BE 705/0038). Both authorities approved the study protocol by considering the animal welfare and the ethics of the research. Access to the Petite Terre Nature Reserve was approved by officers of the Office National des Forêts (ONF, Guadeloupe).

### 2.3. Sample preparation and chemical analysis

Egg yolk, albumen and eggshell were isolated from two eggs collected from each encountered female (for each egg fraction,  $n = 22$  and  $n = 8$  for green and hawksbill turtles, respectively), and the epidermis layer was removed from the dermis layer ( $n = 11$  and  $n = 4$  for green and hawksbill turtles, respectively). Twenty-three PCB congeners (i.e. IUPAC 28, 44, 52, 66 + 95, 70, 87, 95, 101, 110, 118, 128, 138, 149, 153, 156, 170, 180, 183, 187, 194, 195, 206), DDTs ( $p$ ,  $p'$ -DDT,  $p$ ,  $p'$ -DDE,  $pp'$ -DDD), HCHs ( $\alpha$ -HCH,  $\beta$ -HCH, lindane) and chlordecone were determined in egg yolk and dermal tissue according to methods previously described (Multigner et al., 2010; Guillet et al. 2010; Debier et al., 2003). Briefly, four g of fresh samples were freeze-dried with a Benchtop 3L Sentry Lyophilisator (VirTis, New-York, USA). The dry lyophilized samples were weighed in order to determine the water content. The extraction of compounds of interest was performed on 500 mg of freeze dried egg yolk and dermal tissue after addition of 500 mg of anhydrous sodium sulphate with a mixture of  $n$ -hexane, dichloromethane and methanol (5:2:1, v:v:v) using an Accelerated Solvent Extractor ASE (Dionex 200, Sunnyvale, USA) at  $80\text{ }^{\circ}\text{C}$  and under a pressure of 1500 Psi. Before the extraction, 100  $\mu\text{L}$  of an hexanic solution of PCB congener 112 (Dr. Ehrenstorfer®, Augsburg, Germany) was added as a surrogate internal standard at a final concentration of 50 pg/ $\mu\text{L}$  to the samples in order to quantify possible loss of compounds of interest during the extraction and purification procedures. The solvent with extracted fat were collected in pre-weighed vials and evaporated at  $40\text{ }^{\circ}\text{C}$  under a gentle nitrogen flow (Turbovap) using a Turbovap LV (Zymark, Hopkinton, Mass., USA) until a constant weight was obtained and fat content was determined gravimetrically. The residues containing both lipids and OHCs of interest were dissolved into 3 ml of  $n$ -hexane and collected into a test tube. These extracts were subjected to clean-up with 2 mL of sulphuric acid (98%–100%) (Merck, Darmstadt, Germany) in order to remove organic matter (lipids, lipoproteins, glucides). The mixture was homogenized by vortexing for 1 min 5 with a Vibramax 110 (Heidolph, Germany) and centrifuged for 3 min at 2160G at  $10\text{ }^{\circ}\text{C}$  with a JOUAN BR4i centrifuge (Jouan, St-Nazaire, France). The organic phases were collected into a new tube and the sulphuric acid layer was extracted with another volume of 3 ml of  $n$ -hexane. The two resulting organic layers were pooled and evaporated just to dryness using a Visidry evaporator (Supelco, Sigma-Aldrich, St-Louis, USA). Therefore, each sample was dissolved with 120  $\mu\text{L}$  of  $n$ -hexane and split in two equal parts. Chlordecone has been analysed in one of these 60  $\mu\text{L}$  parts after addition of 60  $\mu\text{L}$  of PCB 209 as an injection volume internal standard 100 pg/ $\mu\text{L}$  in  $n$ -hexane (Dr. Ehrenstorfer®, Augsburg, Germany).

The other OHCs were analysed on the second 60  $\mu\text{L}$  part. This one was diluted with  $n$ -hexane to 1 ml prior to a second clean-up performed with Florisil solid phase cartridges (Supelco, Envi-Florisil, Bellefonte, PA). The cartridges were first conditioned with successively 5 ml of acetone, 5 ml of a mixture of acetone–hexane (50:50, V:V) and 12 ml of hexane. The sample was then added to the cartridge. Polar molecules were retained on the Florisil (magnesium-silicate). The test tubes containing the sample were rinsed with 3 ml of hexane and added to the cartridge. Another 3 ml of hexane were finally directly added to the column. The eluate was then evaporated to dryness under a nitrogen flow. Therefore, the extract was solubilized with 60  $\mu\text{L}$  of  $n$ -hexane and 60  $\mu\text{L}$  of Mirex (100 pg/ $\mu\text{L}$  in hexane) as injection volume internal standard (Dr Ehrenstorfer GmbH, Augsburg, Germany). Such an analytical procedure has allowed us to confirm that there was nor Mirex, nor PCB209 in any sample.

The purified extracts were analysed by high-resolution gas chromatography using a Thermo Quest Trace 2000 gas chromatograph equipped with a  $\text{Ni}^{63}$  ECD detector (Thermo Quest, Milan,



**Fig. 1.** Map of Guadeloupe Island in the French West Indies (FWI) and dermis collection from a green (*Chelonia mydas*) marine turtle. Collection sites are indicated in yellow. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Italy) and an autosampler Thermo Quest AS 2000 (Thermo Quest, Milan, Italy). Chlordecone analysis was performed on a 30 m × 0.25 mm (0.25 μm film) DB-XLB capillary column (J&W Scientific, USA) whereas other OHCs were analysed on a 30 m × 0.25 mm (0.25 μm film) DB5ms capillary column (J&W Scientific, USA). The other analytical parameters were described elsewhere (Debier et al., 2003; Multigner et al., 2010). A procedural blank, consisting of OHCs-free and chlordecone-free freeze-dried milk cream (Carlsbourg, Belgium; 30% lipids) was run with each sample series (20 samples) to control the extraction and clean-up procedure. Quality control (QC) was performed by regular analyses of procedural blanks, by the injection of standard and n-hexane blanks. The QC was freeze-dried milk cream enriched with defined concentrations of chlordecone and other OHCs (1–5 ng.g<sup>-1</sup>). Data were recorded with Chromcard 2.2 (Fisons Instruments, Italy) software for Windows. Chlordecone and OHCs were identified on the basis of their respective retention time relative to that of the injection volume internal standard used for quantification (i.e. PCB 209 for chlordecone and Mirex for the OHCs). The linear calibration curve (5–120 pg.μl<sup>-1</sup>) was established with PCBs and pesticides certified solutions (Riedel-de Haën, Seelze, Germany) and a good correlation ( $r > 0.99$ ) was achieved. The chlordecone and other OHCs concentrations in each sample and in the QC was corrected for initial sample weight, and the percentage recovery of the surrogate PCB 112. Recovery rates were always between 70% and 130% according to the requirement (SANCO, 2014; E.C. Council, 2002).

PBDE-congeners (i.e. IUPAC numbers 28, 47, 66, 85, 99, 100, 153, 154, 183) and two methoxylated-PBDEs (i.e. 2'-MeO-BDE-68 and 6-MeO-BDE-47) were determined in three and two egg yolks collected from green and hawksbill turtles, respectively. A gas chromatography-mass spectrometry method was used as previously described (Covaci et al., 2002) and the analysis of standard reference material SRM 1945 (PBDEs in whale blubber) indicated that method accuracy was above 90%.

Essential (i.e. selenium, copper, iron, zinc) and non-essential (i.e. cadmium, lead, mercury) trace elements were determined in egg yolk, albumen and eggshell (for each egg fraction,  $n = 11$  and  $n = 4$  for green and hawksbill turtles, respectively) were analysed separately. A mass spectrometry method after microwave-assisted digestion was used as previously described (Guirlet et al., 2008). Samples for Cd, Cu, Se, Pb and Zn were analysed using an Inductively Coupled Plasma-Mass Spectrometer (ICPMS, Elan DRC II). Samples for mercury were analysed using a Direct Mercury Analyser (DMA Milestones). Parallel to samples, a set of certified control material samples (DOLT-3 liver, National Research Council Canada and Whole Egg Powder Standard Reference Material 8415, National Institute of Standards and Technology) went through each set of analyses to ensure the accuracy and precision of the method (Guirlet et al., 2008). Recoveries for control materials ranged from 90% to 100% for Fe, Cu, Zn and Se and from 103% to 117% for Cd, Pb and Hg.

#### 2.4. Risk assessment

The pollutant concentrations used for risk assessments were calculated for the egg content (i.e. yolk plus albumen). For each egg, the concentrations measured in the yolk and albumen were multiplied by the respective mass of the tissue. The resulting burdens were then summed and divided by the mass of the egg content.

Screening risk assessment SRA was performed for the major OHCs and trace elements measured in green and hawksbill turtle eggs. The method previously described for marine turtles (Lam et al., 2006; Van de Merwe et al., 2009) was used and consisted of calculating the hazard quotients (HQs). For a given pollutant, HQs were computed by dividing the MEC (Measured Egg Concentration) of that pollutant by its predictable no-effect concentration (PNEC). Globally speaking, no studies have reported



such values for marine turtles and PNECs were therefore based on studies on other oviparous vertebrates, such as freshwater turtles and birds (see electronic supporting information and Sup. Table 1). Each species was considered separately in the HQ estimation and HQs were estimated for a best- (i.e.  $HQ_{best}$ ) and worst-case (i.e.  $HQ_{worst}$ ) scenario by considering the minimum (i.e.  $MEC_{min}$ ) and the maximum MEC (i.e.  $MEC_{max}$ ), respectively. HQs were expressed in toxic units (TU); toxic effects should be expected for  $HQs > 1$  TU but are less likely for  $HQs < 1$  TU.

### 2.5. Statistical analyses

The following six classes of OHCs were considered for statistical analyses and only detected compounds were summed for calculating total concentrations: (1) the sum of the 23 PCB congeners,  $\sum PCBs$ ; (2) the sum of the seven major PCB congeners detected in the environment and known as the International Council for the Exploration of the Sea (ICES) set,  $\sum 7ICES$  (i.e. PCBs congeners 28, 52, 101, 118, 138, 153, 180); (3) the sum of the six major non-dioxin-like PCB congeners (NDL-PCBs) detected in the environment and known as the International Council for the Exploration of the Sea (ICES) set,  $\sum 6ICES$  (i.e. PCBs congeners 28, 52, 101, 138, 153, 180); (4) the sum of  $p, p'$ -DDT,  $p, p'$ -DDE and  $p, p'$ -DDD,  $\sum DDTs$ ; (5) the sum of alpha-HCH, beta-HCH and lindane,  $\sum HCHs$ ; and (6) chlordecone. Because NDL-PCB and dioxin-like-PCB (DL-PCBs) congeners show different toxicity mechanisms (Eur-Lex, 2011; Giesy and Kannan, 1998), only NDL-PCBs were considered in the present risk assessments. Therefore, the  $\sum 6ICES$  was used for computations because the  $\sum 7ICES$  contains the DL-PCB 118.  $\sum PBDEs$  and MeO-PBDEs were not subjected to statistical analysis due to the low sample size but results were discussed. Due to the low sample size following analytical procedures, statistical analyses were not conducted on OHC concentrations measured in the hawksbill dermis ( $n = 3$ ) and yolk ( $n = 1$ ). Statistical computations were however possible for chlordecone levels in the hawksbill yolk ( $n = 5$ ).

The R program (2.14.1 version) with the Nondetects and Data Analysis for environmental data package (NADA) handling left-censored data was used for summary statistics as previously recommended (Helsel and Lopaka, 2005; Singh and Nocerino, 2002). The mean, standard error and median were estimated by using the Kaplan–Meier (KM) or regression on order (ROS) models. KM was used for  $\leq 50\%$  of non-detected values (ND) and ROS for  $> 50\%$  of ND. For compounds with 100% detection frequency, variance analysis was performed through the Statistica 9.0 software (StatSoft Inc.). For those with less than 100% detection frequency, the R's NADA package was used for comparisons. Following recommendations for low sample size (Singh and Nocerino, 2002), results were qualified as significant for a  $p$ -value  $< 0.1$ .

Species differences were tested between element (i.e. yolk, albumen and eggshell) and OHC (i.e. yolk and albumen) concentrations (Mann–Whitney test). PCB congeners were grouped by degree of chlorination as follows:  $\sum$  tri- and tetra-chloro PCBs ( $\sum 3-4Cl$  PCBs),  $\sum$  penta-chloro PCBs ( $\sum 5Cl$  PCBs),  $\sum$  hexa-chloro PCBs ( $\sum 6Cl$  PCBs) and  $\sum$  hepta-, octa- and nona-chloro PCBs ( $\sum 7-9Cl$  PCBs). The mean contribution percentage of each group to the  $\sum PCBs$  was then calculated. Difference in percentage contribution was tested in the same tissue (Wilcoxon test). Spearman relationships were tested between pollutant concentrations within a given tissue (i.e. in yolk or albumen or eggshell or dermis) and between tissues (i.e. trace elements: yolk versus albumen versus eggshell; OHCs: yolk versus albumen versus dermis).

Differences in pollutant patterns were investigated through principal component analyses (PCA). The mean percentage contribution of each OHC class to  $\sum OHCs$  was used for investigations in

yolk, albumen and dermis. Likewise, the mean percentage contribution of each trace element (i.e. Se, Cu, Zn, Fe, Cd, Pb and Hg) to  $\sum Elements$  was calculated in yolk, albumen and eggshell. Eigenvalues indicate the amount of variance explained by each principal component (PC). By convention, every PC with a value above 1 is considered as significant and retained in the analysis.

## 3. Results

Concentrations of the usual OHCs and trace elements cited in literature were reported for Guadeloupean green and hawksbill turtles, and compared with previous studies in Tables 1 and 2 (OHCs), and in Table 3 (trace elements). Concentrations were further expressed on a total egg content basis (i.e. yolk plus albumen). All the investigated compounds were reported in electronic supplemental tables (Sup. Table 2 and 3) with chlordecone in a separate table (Table 4).

### 3.1. Pollutant pattern in green and hawksbill marine turtles

In both species,  $\sum PCBs$  was the main OHC group accounting for  $58 \pm 5\%$ ,  $72 \pm 6\%$  and  $73 \pm 6\%$  (mean  $\pm$  SE) of the  $\sum OHCs$  in green turtle yolk, albumen and dermis, respectively. Likewise,  $\sum PCBs$  accounted for 56% (only one sample was available),  $51 \pm 12\%$  and  $69 \pm 9\%$  of the  $\sum OHCs$  in hawksbill turtle yolk, albumen and dermis, respectively. Chlordecone was the second contaminant in both species albumen ( $23 \pm 6\%$  and  $31 \pm 9\%$  for green and hawksbill turtles, respectively) and in hawksbill turtle yolk (29%). Conversely,  $\sum HCHs$  was the second group ( $20 \pm 5\%$ ) in green turtle yolk. Among  $\sum PBDEs$ , BDE-47 and BDE-99 were the only detected congeners in yolk (Table 1). They were in general above the quantification limit (i.e.  $0.018 \pm 0.002$  ng.g<sup>-1</sup> wet weight, QL) in green turtles, but  $< QL$  in hawksbill turtles (Sup. Table 2). 2'-MeO-BDE68 was  $> QL$  in both species. 6-MeO-BDE-47 was  $> QL$  (i.e.  $0.026$   $\mu g.g^{-1}$  w.w.) in green turtles but  $< QL$  in 50% of the hawksbill samples (Sup. Table 2). Toxic elements (i.e. cadmium, lead, mercury) accounted for less than 3% of the  $\sum Elements$  in both species' eggs. Cadmium concentrations were below quantification limits in albumen of both species (i.e.  $0.014$  and  $0.005$   $\mu g.g^{-1}$  w.w., respectively), and in green turtle yolk (i.e.  $0.003$  and  $0.001$   $\mu g.g^{-1}$  w.w., respectively).

PCB congeners were grouped according to their degree of chlorination and the mean contribution percentage of each group was calculated to the  $\sum PCBs$  (Fig. 2). In green turtle yolk,  $\sum 3-4Cl$  PCBs was the main group (Wilcoxon,  $0.0003 \leq p \leq 0.001$ ). In addition,  $\% \sum 5Cl$  PCBs and  $\% \sum 6Cl$  PCBs were higher than  $\% \sum 7-9Cl$  PCBs (Wilcoxon,  $p = 0.019$  and  $0.013$ , respectively). In the albumen of green turtle albumen,  $\sum 3-4Cl$  PCBs was the main group (Wilcoxon,  $p = 0.002-0.047$ ) and  $\% \sum 5Cl$  PCBs was higher than  $\% \sum 7-9Cl$  PCBs (Wilcoxon,  $p = 0.010$ ). In hawksbill turtle albumen,  $\% \sum 3-4Cl$  PCBs was higher than  $\% \sum 5Cl$  PCBs and  $\% \sum 6Cl$  PCBs (Wilcoxon,  $p = 0.031$ ). In green turtle dermis,  $\% \sum 3-4Cl$  was higher than  $\% \sum 7-9Cl$  PCBs (Wilcoxon;  $p = 0.019$ ). No further statistical differences were observed.

### 3.2. Statistical differences between species

Green turtles deposited lower levels of chlordecone, mercury and selenium but higher levels of copper in the yolk than hawksbill turtles (Mann–Whitney;  $p = 0.012$ ,  $0.0011$ ,  $0.012$  and  $0.0132$ , respectively). In addition, green turtles accumulated higher levels of PCBs (i.e.  $\sum PCBs$ ,  $\sum 5Cl$  PCBs,  $\sum 6Cl$  PCBs and  $\sum 7-9Cl$  PCBs) in the albumen than hawksbill turtles (Mann–Whitney;

**Table 1**

Organohalogen compound concentrations (ng.g<sup>-1</sup> w.w.) in eggs collected from marine turtles and crocodiles from different locations, including Guadeloupean green and hawksbill turtles.

Tissue Species Location	<i>n</i> (Reference)	ΣPCBs	ΣHCHs	ΣDDTs	PBDE-47	PBDE-99	ΣPBDEs
		Mean ± SE and/or range *					
Egg yolk							
Green marine turtle, <i>Chelonia mydas</i>							
French West Indies	20 present study	4.47 ± 0.56 0.55 – 8.62	1.47 ± 0.48 <0.004 – 8.08 (90)	0.30 ± 0.05 <0.004 – 0.65 (80)	0.014 – 0.040 <sup>A</sup>	<0.013 – 0.031 <sup>A</sup>	0.021 – 0.071 <sup>A</sup>
Loggerhead marine turtle, <i>Caretta caretta</i>							
Western Florida	11 (Alava et al., 2011)	32.4 ± 14.1 1.54 – 151	0.449 ± 0.017 <0.41 – 1.09	23.8 ± 7.1 2.36 – 74	0.77 ± 0.077 <sup>B</sup> <0.29 – 1.33	0.35 ± 0.063 <sup>B</sup> <0.14 – 0.47	1.08 ± 0.20 <sup>B</sup> <0.14 – 2.56
Eastern Florida	24 (Alava et al., 2011)	372 ± 148 7.13 – 3,010	1.21 ± 0.49 <0.43 – 10.4	136 ± 56 0.78 – 1,030	1.25 ± 0.27 <sup>B</sup> <0.34 – 4.41	0.35 ± 0.078 <sup>B</sup> <0.14 – 2.28	2.43 ± 0.55 <sup>B</sup> <0.16 – 7.82
North Carolina	9 (Alava et al., 2011)	1,460 ± 493 32.9 – 3,500	3.15 ± 1.39 <0.54 – 13.1	694 ± 251 4.97 – 2,170	2.61 ± 0.96 <0.43 – 7.74	1.66 ± 0.13 <0.18 – 13.9	13.5 ± 4.8 0.43 – 37
South Carolina	10 <sup>C</sup> (Keller, 2013)	253.5 ± 93.1 41.2 – 953.9	N.A.	125 ± 71.2 11.9 – 757.7	N.A.	1.3 ± 0.5 0.23 – 4.7	
Hawksbill marine turtle, <i>Eretmochelys imbricata</i>							
French West Indies	1 present study	4.55	0.73	0.51	<0.004 – 0.015 <sup>A</sup>	<0.013 <sup>A</sup>	<0.018 – 0.02 <sup>A</sup>
Egg content							
Green marine turtle, <i>Chelonia mydas</i>							
French West Indies	13 present study	4.57 ± 0.47 2.01 – 7.9 (95)	0.66 ± 0.23 0.14 – 3.13 (81)	0.17 ± 0.04 0.01 – 0.48 (71)	N.A.		
Peninsular Malaysia	55 (van de Merwe et al., 2009)	0.47 ± 0.083 0.15 – 3.69	0.069 ± 0.009 0.013 – 0.23	0.083 ± 0.018 <0.001 – 0.70	N.A.		0.022 ± 0.007 <0.001 – 0.35

(continued on next page)

$p = 0.0046, 0.037, 0.0072$  and  $0.029$ , respectively). Green turtles deposited lower levels of copper, selenium and mercury in the eggshell than hawksbill turtles (Mann–Whitney;  $p = 0.0011, 0.0011$  and  $0.0020$ , respectively).

The PCA supported species separation. In yolk, the first PC explained 47% of the variation associated with mercury and selenium. In eggshell, the first PC explained 54% of the variation associated with mercury and selenium. In albumen, the first PC explained 41% of the variation associated with selenium. The PC analysis based on OHCs did not separate both species but rather some individuals based on %PCBs and %chlordecone.

### 3.3. Pollutant relationships between tissues

Positive correlations were observed between chlordecone levels in dermis and those in yolk (Spearman,  $r^2 = 0.25$ ,  $p = 0.04$ ) and albumen (Spearman,  $p = 0.011$ ,  $r^2 = 0.39$ ). Positive correlations were further observed between levels of Σ7ICES, Σ5CIPCBs and Σ6CIPCBs in dermis and albumen (Spearman;  $p = 0.033, 0.004$  and  $0.002$ ;  $r^2 = 0.30, 0.48$  and  $0.53$ , respectively) and between Σ3-4Cl PCBs and Σ6CIPCBs in these tissues (Spearman;  $p = 0.054$  and  $0.004$ ,  $r^2 = 0.31$  and  $0.49$ , respectively). One negative correlation was observed between the levels of Σ7-9Cl PCBs in dermis and yolk (Spearman,  $p = 0.0427$ ,  $r^2 = 0.25$ ).

Table 1 (continued)

Tissue Species Location	<i>n</i> (Reference)	ΣPCBs	ΣHCHs	ΣDDTs	PBDE-47	PBDE-99	ΣPBDEs
		Mean ± SE and/or range *					
Egg content							
Green marine turtle, <i>Chelonia mydas</i>							
Terengganu, Malaysia	33 (van de Merwe et al., 2010)	0.55 ± 0.055 0.39 – 0.84	0.17 ± 0.007 0.14 – 0.20 <sup>D</sup>	N.A.	0.022 ± 0.002 0.011 – 0.028	0.032 ± 0.004 0.012 – 0.055	0.13 ± 0.008 0.062 – 0.17
Leatherback marine turtle, <i>Dermochelys coriacea</i>							
Juno Beach, Eastern Florida	6 <sup>E</sup> (Stewart et al., 2011)	8.45 ± 3.1 0.44 – 19.9	N.A.	1.87 ± 0.4 0.68 – 3.49	0.47 ± 0.13 0.073 – 0.804	0.077 ± 0.011 <0.018 – 0.11	0.85 ± 0.26 0.12 – 1.64
Hawksbill marine turtle, <i>Eretmochelys imbricata</i>							
French West Indies	1 present study	1.72	0.47	0.19	N.A.		
Morelet's crocodile, <i>Crocodylus moreletii</i>							
Gold Button Lagoon, Belize	17 <sup>F</sup> (Wu et al., 2000)	N.A.	N.A.	N.A. 10.0 – 180.0	N.A.		
New River Lagoons, Belize	7 <sup>F</sup> (Keller et al., 2006)	N.A.	N.A.	N.A. <DL – 110.0	N.A.		
Gold Button Lagoon, Belize	72 <sup>G</sup> (Wu et al., 2006)	N.A.	N.A.	61 ± 1.4 (DDE) 291 ± 9.2 (DDE) 13 ± 0.6 (DDT) 22 ± 0.5 (DDD)	N.A.		
New River Lagoons, Belize	25 <sup>H</sup> (Wu et al., 2006)	N.A.	N.A.	34 ± 8 (DDE) <DL (DDT) <DL (DDD)	N.A.		

N.A., no available data. \*: The <-sign means that values were below the quantification or detection limit, i.e. QL and DL respectively. In the present study, a mean QL was reported and the percentage of samples above the QL was indicated in brackets. [P.S.]: the present study. A: PBDE analyses were only performed in three and two egg yolks collected from green and hawksbill marine turtles, respectively. B: PBDEs analyses were performed in 10 and 19 eggs from western and eastern Florida, respectively. C: Results were converted from lipid to wet weight by considering a lipid content of 30% in yolk. D: Results were only available for  $\gamma$ -HCH. E: The sample number refers to sampled nests. Authors mentioned that three to six eggs were collected and analysed per nest. F: Results were only available for DDE. G: 72 eggs from three clutches were collected. For DDE, the lowest and highest mean ± SD were reported. For DDT and DDD, only one mean ± SD was available. H: One clutch of 25 eggs was analysed.

### 3.4. Risk assessments

Potential risk was characterized for a given chemical for both developing embryos through the calculation of Hazard Quotients HQs (Table 5). HQs varied from <0.1 to 3.0 for OHCs, and from <0.1 to 15.0 for trace elements. HQs > 1 were reported for *p,p'*-DDE (HQ<sub>worst</sub> = 3.0 for green turtle embryos), for the lowest cadmium PNEC (HQ<sub>best/worst</sub> = 1.6/5.1 for green turtle embryos; HQ<sub>best/worst</sub> = 1.4/3.6 for hawksbill turtle embryos), the lowest selenium PNEC (HQ<sub>worst</sub> = 1.3 for green turtle embryos; HQ<sub>best/worst</sub> = 1.8/5.7 for hawksbill turtle embryos) as well as for

mercury (HQ<sub>worst</sub> = 4.4 for green turtle embryos; HQ<sub>worst</sub> = 15.0 for hawksbill turtle embryos).

## 4. Discussion

### 4.1. Pollutant exposure in nesting green and hawksbill turtles from Guadeloupe

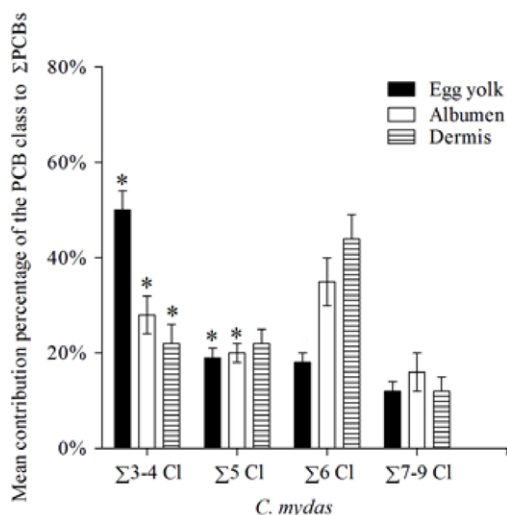
Egg and dermis samples were collected from 11 green and four hawksbill females. ΣPCBs and chlordecone were the main contaminant groups in the dermis of Guadeloupean green and hawksbill marine turtles (Tables 2 and 4). Contaminants detected

**Table 2**

Organohalogen compound concentrations (ng.g<sup>-1</sup> w.w.) in eggs collected from marine turtles and crocodiles from different locations, including Guadeloupean green and hawksbill turtles.

Species Location	<i>n</i> [Reference]	ΣPCBs	ΣHCHs	ΣDDTs
		Mean ± SE and/or range *		
Green marine turtle, <i>Chelonia mydas</i>				
French West Indies	10 present study	291.94 ± 116.54 12.72 – 1,229.7	42.49 ± 32.91 <0.56 – 336.88 (80)	26.63 ± 19.11 <0.56 – 182.81 (40)
Hawksbill marine turtle, <i>Eretmochelys imbricata</i>				
French West Indies	3 present study	N.A. 39.37 – 107.08	<0.56	N.A. <0.56 – 47.7 (66)
Morelet's crocodile, <i>Crocodylus moreletii</i>				
Gold Button Lagoon, Belize	9 (DeBusk, 2001)	N.A.		<DL – 389.0 (DDE) <DL – 22.0 (DDD) <DL – 152.0 (DDT)

N.A., no available data. \*: The <-sign means that values were below the quantification or detection limit, i.e. QL and DL respectively. In the present study, a mean QL was reported and the percentage of samples above the QL was indicated in brackets. [P.S.], the present study.



**Fig. 2.** Mean contribution percentage (±SE) of each PCB group to the ΣPCBs in egg yolk, albumen and dermis of green (*Chelonia mydas*) marine turtles from Guadeloupe. Σ3-4 Cl, sum of tri- and tetra-PCBs; Σ5 Cl, sum of penta-PCBs; Σ6 Cl, sum of hexa-PCBs; Σ7-9 Cl, sum of hepta-, octa- and nona-PCBs. Statistical differences are indicated by the \$ and \*-sign. \$: Σ3-4Cl PCBs was the main group in the yolk and albumen. In addition, %Σ3-4Cl PCBs was higher than %Σ7-9Cl PCBs in their dermis; \*: %Σ5Cl PCBs was higher than %Σ7-9Cl PCBs in the yolk and albumen. In addition, %Σ6Cl PCBs was higher than %Σ7-9Cl PCBs in the yolk.

in turtle eggs reflect maternal deposition during egg formation (Tables 1, 3 and 4). Σ3-4Cl PCBs dominated in green turtle egg yolk and albumen indicating that less chlorinated PCBs were more readily transferred than more chlorinated PCBs ( $p \leq 0.001$ , Fig. 2) during egg production. The PCB pattern observed in hawksbill turtle eggs differed from that observed in green turtle eggs. The hawksbill albumen tended to have lower level of more chlorinated PCBs (i.e. Σ6Cl and Σ7-9Cl PCBs) but higher level of Σ3-4Cl PCBs than yolk (Sup. Table 2).

Previous studies in marine turtles have reported correlations between chemical levels in females and their eggs (Stewart et al., 2011; Van de Merwe et al., 2010). In Guadeloupe, dermis samples were collected from green and hawksbill marine turtle females

and analysed for OHCs. Correlations were then tested between OHC concentrations measured in dermis and eggs (i.e. yolk and albumen). A positive relationship was observed between chlordecone levels in green turtle yolk and dermis while a negative relationship was observed for Σ7-9Cl PCBs levels between these two tissues. Many more positive relationships were observed between pollutant levels in the albumen and dermis (i.e. different groups of PCBs and chlordecone), suggesting that pollutants were similarly accumulated in these two tissues. Since chemical levels in dermis were expected to reflect exposure at the marine turtle foraging area (Barrow, 2006; Seminoff et al., 2006; Wallace et al., 2006), albumen may likewise reflect chemical exposure at the females' feeding ground.

Egg yolk and albumen are produced at different times in marine turtles. Vitellogenesis is initiated eight to 10 months prior to the nesting season (i.e. at the foraging ground) and completed before females migrate to their nesting ground. There, and prior to each egg laying event, albumen and eggshell are deposited completing the egg formation (Miller et al., 2003). The accumulation pattern of PCBs in Guadeloupean marine turtle yolk and albumen was therefore expected to differ. Instead, a similar pattern was observed in the green turtle eggs (Fig. 2) suggesting that females used a similar energy source for producing their yolk and albumen. These results suggested that females fed during the nesting season (i.e. income breeders), likely on seagrass meadows and reefs present in the Guadeloupe archipelago (Kamel and Delcroix, 2009; Tucker and Read, 2001). This hypothesis is supported by the similar accumulation pattern of PCBs in the egg fractions and dermis (Fig. 2) and by the presence of resident turtles reported in Guadeloupe (Kamel and Delcroix, 2009).

The tetra-BDE-47 and penta-BDE-99 were the only PBDE congeners quantifiable in Guadeloupean turtle eggs (Table 1) and may be consistent with the past use of a penta-BDE mixture in the investigated area (Birnbbaum and Staskal, 2004). A low exposure of the Guadeloupean marine turtles to PBDEs may be expected, as well as a preferential transfer of lower-brominated PBDEs during vitellogenesis and/or their ability in metabolizing such compounds. Among the two MeO-PBDE analogues detected in eggs, 2'-MeO-BDE-68 was in higher proportion than 6-MeO-BDE-47 (Sup. Table 1), indicating sponges and associated organisms



**Table 3**Trace element concentrations ( $\mu\text{g.g}^{-1}$  w.w.) in marine turtle yolk, albumen and eggshell collected from different locations.

Tissue species location	n [Reference]	Copper Mean $\pm$ SE and/or range*	Selenium	Cadmium	Lead	Mercury
Egg yolk						
Green marine turtle, <i>Chelonia mydas</i>						
French West Indies	12 Present study	$0.59 \pm 0.02$ 0.50–0.67	$0.17 \pm 0.026$ 0.063–0.30	$<0.003$ $<0.003$ –0.008 (18)	$0.010 \pm 0.0014$ 0.0055–0.022	$0.0020 \pm 0.0003$ 0.0010–0.0045
Haha-Jimma Island, Japan	2 (Sakai et al., 2000)	0.63	N.A.		$<0.03$	2.51
Hong Kong, China	30 (Lam et al., 2006)	$0.34 \pm 0.036$ 0.17–0.77	$3.5 \pm 0.6$ 1.3–7.6	$<0.0005$	$0.049 \pm 0.008$ 0.025–0.14	$0.0015 \pm 0.00013$ 0.00093–0.0023
Loggerhead marine turtle, <i>Caretta caretta</i>						
Cape Ashizuri, Japan	6 (Sakai et al., 2000, 1995)	$1.57 \pm 0.073$ 1.48–1.68	N.A.	$0.026 \pm 0.007$ 0.19–0.035	$<0.03$	$12.1 \pm 3.41$ 0.008–0.016
Hawksbill marine turtles, <i>Eretmochelys imbricata</i>						
French West Indies	4 Present study	$0.49 \pm 0.027$ 0.41–0.53	$1.37 \pm 0.19$ 1.07–1.89	$<0.003$ $<0.003$ –0.005(50)	$0.017 \pm 0.002$ 0.012–0.022	$0.017 \pm 0.002$ 0.013–0.024
Olive ridley marine turtle, <i>Lepidochelys olivacea</i>						
Oaxaca, Mexico	250 <sup>A</sup> (Páez-Osuna et al., 2011, 2010a,b)	$0.83 \pm 0.55$	N.A.	$0.09 \pm 0.038$	$0.3 \pm 0.038$	$0.011 \pm 0.0038$
Egg content						
Green marine turtle, <i>Chelonia mydas</i>						
French West Indies	12 Present study	$0.68 \pm 0.045$ 0.50–1.06	$0.21 \pm 0.038$ 0.076–0.21	$<0.008$	$0.013 \pm 0.0012$ $<0.012$ –0.025	$0.011 \pm 0.003$ 0.0047–0.044
Markets in Peninsular Malaysia	55 (Van de Merwe et al., 2009)	$0.53 \pm 0.023$ 0.056–1.073	$0.46 \pm 0.026$ 0.049–0.84	$0.009 \pm 0.001$ $<0.01$ –0.029	$0.031 \pm 0.003$ $<0.05$ –0.124	N.A.
Egg content						
Haha-Jimma Island, Japan	2 (Sakai et al., 2000)	0.78	N.A.		$<0.03$	1.35
Loggerhead marine turtle, <i>Caretta caretta</i>						
Cape Ashizuri, Japan	6 (Sakai et al., 2000, 1995)	$1.05 \pm 0.2$ 0.77–1.31	N.A.	$0.013 \pm 0.004$ 0.008–0.015	$<0.03$	$5.54 \pm 1.57$ 0.0038–0.0074
Hawksbill marine turtle, <i>Eretmochelys imbricata</i>						
French West Indies	3 Present study	0.13–0.50	0.61–1.94	$<0.008$	$<0.012$ –0.015	0.009–0.15
Olive ridley marine turtle, <i>Lepidochelys olivacea</i>						
Curtis Island, Australia	60 (Ikonomopoulou et al., 2011)	N.A.	$0.30 \pm 0.002$		$<0.05$	
Albumen						
Green marine turtle, <i>Chelonia mydas</i>						
French West Indies	12 Present study	$0.85 \pm 0.07$ 0.56–1.20	$0.27 \pm 0.06$ $<0.096$ –0.81 (91)	$<0.014$ $<0.014$ –0.018 (8)	$0.019 \pm 0.0005$ $<0.019$ –0.019 (36)	$0.018 \pm 0.0035$ 0.011–0.14
Haha-Jimma Island, Japan	2 (Sakai et al., 2000)	0.16	N.A.		$<0.03$	0.05
Hong Kong, China	30 (Lam et al., 2006)	$0.063 \pm 0.012$ 0.025–0.18	$0.27 \pm 0.058$ 0.081–0.73	$<0.0005$	$0.0047 \pm 0.001$ $<0.00001$ –0.014	$0.0001 \pm 0.00003$ $<0.00001$ –0.0004
Loggerhead marine turtle, <i>Caretta caretta</i>						
Cape Ashizuri, Japan	6 (Sakai et al., 2000, 1995)	$0.13 \pm 0.083$ 0.034–0.24	N.A.	$<0.01$	$<0.03$	$0.49 \pm 0.24$ $0.0005 \pm 0.0002^B$ 0.0001–0.0008
Hawksbill marine turtle, <i>Eretmochelys imbricata</i>						
French West Indies	3 Present study	0.63–1.09	3.15–6.05	$<0.014$ (0)	$<0.019$ –0.024 (50)	0.013–0.025

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Table 3 (continued)

Tissue species location	n [Reference]	Copper Mean $\pm$ SE and/or range*	Selenium Mean $\pm$ SE and/or range*	Cadmium Mean $\pm$ SE and/or range*	Lead Mean $\pm$ SE and/or range*	Mercury Mean $\pm$ SE and/or range*
Albumen						
Olive ridley marine turtle, <i>Lepidochelys olivacea</i>						
Oaxaca, Mexico	250 <sup>A</sup> (Páez-Osuna et al., 2011, 2010a,b)	0.095 $\pm$ 0.078	N.A.	0.0059 $\pm$ 0.0024	0.029 $\pm$ 0.0054	0.00003 $\pm$ 0.00002
Eggshell						
Green marine turtle, <i>Chelonia mydas</i>						
French West Indies	12 Present study	1.63 $\pm$ 0.11 0.90–2.21	0.15 $\pm$ 0.004 <0.14–0.19 (45)	0.083 $\pm$ 0.005 0.063–0.13	0.006 $\pm$ 0.0008 <0.0076–0.009 (45)	0.0018 $\pm$ 0.0002 0.0008–0.003
Haha-Jimma Island, Japan	2 (Sakai et al., 2000)	4.74	N.A.		<0.03	1.20
Hong Kong, China	30 (Lam et al., 2006)	1.3 $\pm$ 0.33 0.24–4.2	2.5 $\pm$ 0.83 0.45–11.0	0.016 $\pm$ 0.010 <0.0005–0.16	0.11 $\pm$ 0.024 0.029–0.28	0.00061 $\pm$ 0.00016 0.00009–0.0016
Loggerhead marine turtle, <i>Caretta caretta</i>						
Cape Ashizuri, Japan	6 (Sakai et al., 2000, 1995)	5.57 $\pm$ 0.767	N.A.	<0.01	<0.03	4.05 $\pm$ 1.31
Hawksbill marine turtle, <i>Eretmochelys imbricata</i>						
French West Indies	4 Present study	2.64 $\pm$ 0.14 2.41–3.03	1.39 $\pm$ 0.16 1.10–1.75	0.07 $\pm$ 0.004 0.065–0.083	<0.0076 <0.0076–0.016(25)	0.0033 $\pm$ 0.006 0.0023–0.0048
Olive ridley marine turtle, <i>Lepidochelys olivacea</i>						
Oaxaca, Mexico	250 <sup>A</sup> (Páez-Osuna et al., 2011, 2010a,b)	3.07 $\pm$ 1.07	N.A.	0.19 $\pm$ 0.037	0.43 $\pm$ 0.08	0.0036 $\pm$ 0.00012

N.A., no available data. \*: The <-sign means that pollutant concentrations were below the reported QL. The percentage of samples above the QL was reported in brackets. [P.S.], the present study. A: Ten eggs were collected from 25 nests and randomly grouped by five to obtain two pools of eggs per nest. Concentrations were converted from dry to wet weight by considering the moisture reported in the source paper, i.e. 62.5%, 97.3% and 59% for respectively the yolk, albumen and eggshell. B: Two different mercury levels were reported in the source paper but it is probably a miscalculation because a 1000-factor differentiated these values and the same turtles were considered during both studies (six females sampled in April–May 1990 at Cape Ashizuri, Japan).

Table 4

Chlordecone concentrations (ng.g<sup>-1</sup> w.w.) in FWI wildlife including Guadeloupean green and hawksbill marine turtles.

Species	Location	References	Tissue (n)	Concentration (ng.g <sup>-1</sup> w.w.) Mean ± SE and/or range*
Marine organisms				
Green marine turtle, <i>Chelonia mydas</i>	Guadeloupe	Present study	Egg content (16)	0.87 ± 0.25 <0.0035–2.83
			Dermis (10)	83.5 ± 41.6 <0.56–378.0
Hawksbill marine turtle, <i>Eretmochelys imbricata</i>	Guadeloupe	Present study	Egg content (5)	1.34 ± 0.40 0.33–2.24
			Dermis (3)	N.A. <0.56–26.7
American eel, <i>Anguilla rostrata</i>	Grande-Anse River, Guadeloupe	(Coat et al., 2011)	Total flesh (2)	5863 <sup>A</sup>
Surgeon fish, <i>Oreochromis sp.</i>	François Bay, Martinique	(Coat et al., 2006)	Muscle (10)	4.1 <sup>A</sup>
Spiny lobster, <i>Melanoides tuberculata</i>	François Bay, Martinique	(Coat et al., 2006)	Muscle (10)	1.2 <sup>A</sup>
			Total flesh (30)	13.0–31.0 <sup>A</sup>
Freshwater organisms				
Wild red tilapia, <i>Oreochromis sp.</i>	Lézarde River, Martinique	(Coat et al., 2006)	Muscle (20)	196–386 <sup>A</sup>
Red-rimmed melania, <i>Melanoides tuberculata</i>	Grande-Anse River, Guadeloupe	(Coat et al., 2011)	Total flesh (1043 and 92) <sup>B</sup>	1008 and 3570 <sup>B</sup>
River goby, <i>Awaous banana</i>			Total flesh (15 and 12) <sup>B</sup>	1350 and 12,366 <sup>B</sup>

N.A., No available data. \*: The <-sign means that values were below the reported mean QL. [P.S.], the present study. A: Average residue levels were reported in the source paper; B: Average residue levels were reported in the source paper for samples collected during the dry and wet season.

as the main source of MeO-PBDEs (Amorocho and Reina, 2007; Malmvärn et al., 2008; Vetter and Janussen, 2005).

Chlordecone was determined in the dermis of both Guadeloupean marine turtle species as well as in their eggs (Table 4). The high proportion of chlordecone compared to other OHCs in hawksbill turtle yolk and albumen may indicate that females fed on a diet exposed to chlordecone and significantly deposited it into their

eggs. This could further indicate that hawksbill females foraged around Guadeloupe Island since that chemical was mostly used in that region (Le Deaut and Procaccia, 2009). Hawksbill turtles showed higher chlordecone levels in their eggs but lower levels in their dermis than green turtles (Table 4). Different patterns in chlordecone accumulation could be explained by the age of the females, with the older individuals accumulating more chemical in

**Table 5**

Screening risk assessment (SRA) for Guadeloupean green (*Chelonia mydas*) and hawksbill (*Eretmochelys imbricata*) marine turtles. Summary of best- and worst-case hazard quotients (HQ).

Compound	Species	MEC <sub>min</sub> <sup>*</sup>	MEC <sub>max</sub> <sup>*</sup>	PNEC	HQ <sub>best</sub>	HQ <sub>worst</sub>
Trace elements (μg.g <sup>-1</sup> w.w.)						
Selenium	<i>C. mydas</i>	0.08	0.44	0.34 <sup>A</sup>	0.2	<b>1.3</b>
	<i>E. imbricata</i>	0.61	1.94		<b>1.8</b>	<b>5.7</b>
	<i>C. mydas</i>	0.08	0.44	6 <sup>B</sup>	<0.1	0.3
	<i>E. imbricata</i>	0.61	1.94		0.1	0.3
Mercury	<i>C. mydas</i>	0.005	0.04	0.01	0.5	<b>4.4</b>
	<i>E. imbricata</i>	0.009	0.15		0.9	<b>15.0</b>
Lead	<i>C. mydas</i>	0.009	0.03	1	<0.1	<0.1
	<i>E. imbricata</i>	0.007	0.015		<0.1	<0.1
Cadmium	<i>C. mydas</i>	<0.003	0.007	0.0014 <sup>C</sup>	<b>1.6</b>	<b>5.1</b>
	<i>E. imbricata</i>	<0.003	0.005		<b>1.4</b>	<b>3.6</b>
	<i>C. mydas</i>	<0.003	0.007	0.013 <sup>D</sup>	0.2	0.6
	<i>E. imbricata</i>	<0.003	0.005		0.2	0.4
OHCs (ng.g <sup>-1</sup> w.w.)						
<i>p, p'</i> -DDE	<i>C. mydas</i>	<0.004	0.1	0.033	0.1	<b>3.0</b>
	<i>E. imbricata</i> **	<0.004	<0.004		0.1	0.1
Σ 3-4Cl PCBs <sup>E</sup>	<i>C. mydas</i>	1.08	2.71	26	<0.1	0.1
	<i>E. imbricata</i> **	<0.003	0.18	68	<0.1	<0.1
Chlordecone	<i>C. mydas</i>	<0.003	2.83	68	<0.1	<0.1
	<i>E. imbricata</i> **	0.64	2.04		<0.1	<0.1

<sup>\*</sup>: The SRA was performed by considering the minimum and maximum measured egg concentrations of pollutants (i.e. MEC<sub>min</sub> and MEC<sub>max</sub>, respectively). Concentrations were calculated for the whole egg content (i.e. yolk plus albumen). The <-sign means that concentrations were below the reported QL. This QL was indicative and calculated by taking the mean value of all samples. <sup>\*\*</sup>: Only one hawksbill egg was available for the SRA. A and B: PNECs were estimated for the most sensitive<sup>(A)</sup> and tolerant<sup>(B)</sup> bird species, mallard ducks and American avocets, respectively. C and D: PNECs considered effects on eggshell thinning<sup>(C)</sup> and hatchling survival<sup>(D)</sup>. E: PNECs included synergic effects of tri- and tetra-chlorinated congeners. The sum of all tri- and tetra-PCBs was used for the present MECs.

their body than the younger ones. Marine turtles generally show a remigration interval of 2–3 years whereas some Guadeloupean hawksbill turtles have been reported as being annual breeders (Lutz et al., 2003). Therefore, such behaviour may limit the retention of chlordecone in the females' body and favour the elimination of chlordecone through reproduction. In addition, the spongi- givory habits of hawksbill turtles may by an additional field of explanation. Albumin is an important protein for wildlife and is mainly retained in the blood compartment (i.e. ~40% of the protein pool) while the remaining fraction is stored in muscle and skin (Rothschild et al., 1962; Quinlan et al., 2005). In the albumen, the albumin protein is present as ovalbumin and in a smaller amount as conalbumin (i.e. >50% and ~15% of the total protein, respectively). Both forms are involved in element deposition into the egg white but ovalbumin shows more specific binding activity for some elements such as selenium (Richards, 1997).

#### 4.2. Comparison with literature

To the authors' knowledge, the present study was the first to examine levels of OHCs and trace elements in green and hawksbill marine turtles nesting in Guadeloupe (FWI). For the first time, levels of OHCs were reported in hawksbill turtle eggs as was chlordecone in marine turtle tissues. Both Guadeloupean species tended to experience quite different exposure in their foraging ground. Green turtles may globally cope with a higher pollutant threat than hawksbill turtles (Tables 1 and 3). Due to the low sample size for hawksbill turtle tissues, statistical comparisons of OHC concentrations (chlordecone excluded) were only computed for albumen and suggested a higher Σ PCBs exposure for green turtles.

Overall, Guadeloupean marine turtle species may experience lower exposure than the loggerhead *Caretta caretta*, olive ridley *Lepidochelys olivacea* and leatherback *Dermochelys coriacea* marine turtles (Tables 1 and 3). Conversely, they may both deal with higher threats associated with OHC exposure than other green turtle populations (Table 1) but quite similar trace element exposure

compared to other green colonies (Table 3). More specifically, Guadeloupean green and hawksbill turtles accumulated lower levels of Σ PCBs and Σ DDTs in yolk than Florida loggerhead turtles (Alava et al., 2011) (Table 1). They concentrated Σ HCHs in lower levels than North Carolina loggerheads but in higher levels than the Western and Eastern Florida populations. On an egg content basis, both Guadeloupean species accumulated fewer Σ PCBs and Σ DDTs than Florida leatherback turtles (Stewart et al., 2011). Conversely, they both stored higher levels of Σ PCBs and Σ HCHs than green turtle populations from Peninsular Malaysia (Van de Merwe et al., 2010, 2009). The PDBE accumulation pattern in Guadeloupean marine turtle eggs (i.e. BDE-47 to BDE-99 ratio, ~2:1) was comparable to patterns observed in West Florida loggerhead turtles (Alava et al., 2011) but different to those in Malaysia green turtles (Van de Merwe et al., 2010), North Carolina and Eastern Florida loggerhead turtles (Alava et al., 2011). Then, both Guadeloupean species accumulated lower levels of non-essential elements (i.e. cadmium, lead and mercury) than loggerhead and olive ridley turtles. Conversely, these levels were quite similar to those reported for other green turtle populations and for flatback turtles, *Natator depressus* (Table 3). Studies measuring OHCs in dermal tissue are lacking in marine turtles and few pollutants were determined in their reptilian species. In the Morelet's crocodile *Crocodylus moreletii* (DeBusk, 2001), the dermal concentrations of DDTs were higher than those reported for the turtle dermis (present study, Table 2).

The feeding behaviour of green and hawksbill turtles (i.e. seagrass and sponge feeders, respectively Bjørndal, 1985; Meylan, 1988) may account for their low pollutant levels compared to carnivorous loggerhead turtles and crocodiles. The higher selenium levels in Guadeloupean hawksbill turtle eggs than in Guadeloupean green turtle eggs may be likewise due to the food habits of species. Sponges were indicated to strongly depend on selenium for their growth (Müller et al., 2005) while low selenium content was globally reported in seagrasses (Lewis and Devereux, 2009). Chinese green turtles accumulated higher selenium levels (i.e. eggshell and yolk) than other populations, and Guadeloupean

green turtles accumulated higher OHC levels than other green turtle colonies. This suggested that the location of the feeding ground may significantly influence the chemical deposition into tissues. Guadeloupean marine turtles may use foraging ground exposed to lower selenium levels than Chinese green turtles, while Guadeloupean green turtles may feed on ground exposed to higher OHC levels than other green colonies. The post-breeding migratory pattern used by both Guadeloupean species is still poorly understood. Available data reported that hawksbill females may preferentially forage in Puerto Rico ( $22.0 \pm 19.6\%$  of the population) or even in Guadeloupe (Kamel and Delcroix, 2009) while green turtles may feed in the East Caribbean region (Bass et al., 2006).

Generally speaking, little information has been reported about chlordecone levels in wildlife and, unfortunately, no information was available on other marine turtle populations to compare with our data. Nevertheless, chlordecone contamination has been reported in aquatic organisms in Guadeloupe and Martinique (Table 4). In marine fish, levels ranged from 1.2 to 12,266 ng.g<sup>-1</sup> w.w. and from 196 to 386 ng.g<sup>-1</sup> w.w. in freshwater fish. In marine crustaceans, levels ranged from 13.0 to 31.0 ng.g<sup>-1</sup> w.w. and from 1008 to 3570 ng.g<sup>-1</sup> w.w. in freshwater molluscs (Table 4). Obviously, both Guadeloupean marine turtle species concentrated less chlordecone in their eggs than other species, except for the surgeon fish from Fort-de-France, Martinique (Table 4, Coat et al., 2011, 2006). In the marine turtles' dermis, levels were closer to those reported for organisms from Martinique (i.e. freshwater and marine fish, and marine crustaceans) than to those indicated for Guadeloupean wildlife (Table 4, Coat et al., 2011, 2006).

#### 4.3. Risk assessments for Guadeloupean marine turtle embryos

Pollutants were associated with oxidative stress and immune impairments in free-ranging marine turtles (Keller et al., 2006; Komoroske et al., 2011; Labrada-Martagón et al., 2011). Blood was commonly used for assessing such relationships between contaminant levels and physiological response in individuals. However, collecting blood can be difficult in field conditions because of the lack of appropriate expertise of the investigators and/or accessibility of the investigated body area. In a global context of marine turtle conservation and management, there is therefore critical need for developing nondestructive sampling techniques that could be easily put into routine practice. The dermal tissue was previously used for providing information on the foraging ground used by marine turtles (Kamel and Delcroix, 2009; Lemons et al., 2011; Seminoff et al., 2009). Dermis has advantages over blood sampling as it is easier to collect and requires less practical skill. Because the dermis tissue is vascularized and contains lipids, albeit low levels, it was expected to accumulate lipophilic compounds (Alibardi and Toni, 2006) and represent a suitable method for assessing chemical exposure in free-ranging marine turtles. Organic pollutants (e.g. PCBs and chlordecone) were successfully detected in the dermis samples collected from Guadeloupean green and hawksbill turtles (Sup. Table 2). The biological significance of such exposure could not be determined for Guadeloupean marine turtles but associated harmful effects cannot be ruled out. Previous studies suggested a higher prevalence of fibropapillomatosis, an herpes-virus-associated disease in marine turtles from heavily polluted coastal areas (Aguirre and Lutz, 2004; Herbst and Klein, 1995). Guadeloupean marine turtles accumulated pollutants and transferred them into their eggs, arousing concern about chemical embryotoxicity. The risk of Guadeloupean marine turtle embryos to pollutant exposure was assessed through an SRA (Table 5). Assessment indicated that selenium, mercury and cadmium exposure may represent a threat for the developing marine

turtle embryos. The selenium PNECs were based on no-observed-adverse-effect-levels (NOAELs) set for the most sensitive and tolerant bird species to selenium exposure, mallard ducks (*Anas platyrhynchos*) and American avocets (*Recurvirostra Americana*) (Janz et al., 2010), respectively. If Guadeloupean marine turtles are as sensitive as mallards, selenium may reduce the embryos' viability in both species and with a higher probability for hawksbill turtles ( $HQ_{\text{worst}} = 5.7$ ) than for green turtles ( $HQ_{\text{worst}} = 1.3$ ). If marine turtles are as tolerant as avocets, the measured selenium levels may be harmless for both species embryos. This latter scenario may be most likely considering the high hatching rate of both species in Guadeloupe (Masson, 2013). In a worst-case scenario, mercury could induce embryo deformities and/or reduce the survival of green ( $HQ_{\text{worst}} = 4.4$ ) and hawksbill ( $HQ_{\text{worst}} = 15.0$ ) turtle embryos. Similarly, embryos of both species could be impacted by Cd. Compared to previous studies, Guadeloupean green turtle embryos may be exposed to a lower selenium threat than those from Peninsular Malaysia and Hong Kong ( $HQ_{\text{worst}} = 2.5$  and 24.5, respectively Lam et al., 2006; Van de Merwe et al., 2009). As for hawksbill turtle embryos, they may cope with a higher selenium threat than Malaysian green turtle embryos but with a lower threat than Chinese turtle embryos. Cadmium HQs were only reported for Malaysian green turtles ( $HQ_{\text{worst}} = 0.1$ ) (Van de Merwe et al., 2009) and indicated a lower associated risk than for both Guadeloupean marine turtle species.

Unlike Van de Merwe et al. (2009) who based its  $p$ ,  $p'$ -DDE PNEC on a green turtle study (Podreka et al., 1998), the  $p$ ,  $p'$ -DDE PNEC used in the present SRA was based on results from an American alligator *Alligator mississippiensis* study (Milnes et al., 2005). The  $p$ ,  $p'$ -DDE levels reported by Podreka et al. (1998) in eggs (i.e.  $\sim 543$  ng.g<sup>-1</sup>) were considered as not being of biological relevance since they exceeded those reported for marine turtle eggs (Keller, 2013). Therefore, these authors probably missed adverse effects associated with  $p$ ,  $p'$ -DDE exposure, especially considering that such effects tended to increase with decreasing dose (Willingham, 2004). Milnes et al. (2005) reported lower  $p$ ,  $p'$ -DDE levels that induced endocrine disruptions in alligators (i.e.  $\sim 0.33$  ng.g<sup>-1</sup>). In the present study, assessment indicated that the highest  $p$ ,  $p'$ -DDE levels could bias the sex determination of Guadeloupean green turtle embryos ( $HQ_{\text{worst}} = 3.0$ , Table 5).

## 5. Conclusions

The present study provides baseline pollutant concentrations in green and hawksbill marine turtle populations nesting in Guadeloupe. The present results are thus proposed as reference values for future ecotoxicological investigations in marine turtles from the Caribbean region and worldwide. Compared to other marine turtle populations, both Guadeloupean green and hawksbill turtles tended to accumulate higher pollutant levels than other green turtle colonies but may globally cope with a lower exposure than other populations.

A broad range of chemicals were detected and some of them (i.e.  $p$ ,  $p'$ -DDE, cadmium, mercury and selenium) could be harmful for Guadeloupean green and hawksbill turtle embryos. However, it is becoming clear that Guadeloupean marine turtle embryos were exposed to multiple contaminants that could act synergistically and/or produce additive effects resulting in a more significant threat than estimated through the present SRA. Assessing the marine turtle threat to pollutant exposure is worthwhile in a conservation and management context, and should assist decision-makers to prioritize action plans.



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## Appendix A. Supplementary data

Supplementary material related to this article can be found online at <http://dx.doi.org/10.1016/j.rsma.2015.09.004>.

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